PATENT
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Inventor:	Danenberg, Kathleen D.	Art Unit:	1637
Serial No.:	09/842,111	Examiner:	Jeffrey Fredman
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For:	METHOD OF DETERMINING DIHYDROPYRIMIDINE DEHYDROGENASE GENE EXPRESSION		

Assistant Commissioner of Patents
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DECLARATION UNDER 37 CFR § 1.132

I, Kathleen D. Danenberg, do declare and state:

1. I am a citizen of the United States and reside at 3367 Rubio Crest Drive, Altadena, CA 91001.

I am presently President and CEO at Response Genetics, Inc. in Los Angeles, CA 90033.

I received a B.S. in Biochemistry in 1975 from the University of Wisconsin, Madison, WI. Following graduation from the University of Wisconsin, I was a Research Specialist in the Department of Biochemistry at the University of Wisconsin until 1976. I then worked as a Laboratory Specialist at the University of Southern California in the Department of Cancer Research until 2000.

In 2000, I formed Response Genetics, Inc. and my title was Vice President, Chief Scientific Officer until I advanced to my present position in 2002.

I have 29 years of academic and industrial experience in the areas of enzymology, molecular biology and gene expression, during which time I have been the author of 87 articles related to enzymology and gene expression as well as an inventor on ten U.S. patents and more than thirteen pending U.S. patent applications.

2. In my present position as President and CEO, I supervise the development of new methods of determining gene expression based on isolation of RNA from fixed or frozen tissues. Furthermore, I also am responsible for developing methods that relate the expression of various gene markers to determining the proper chemotherapetic regimens for patients. Additionally, to ensure that the development of these methods of determining gene expression reach fruition, I am responsible for developing methods for optimizing primers with regard to specificity.

3. I am the named inventor of the above-mentioned application.

4. I have reviewed and understand the contents of the 09/842,111 patent application. I have also reviewed the Office action dated July 2, 2004, as well as the references cited therein.

As President of Response Genetics, I initiated and supervised the preparation and development of oligonucleotide primers that allow accurate assessment of DPD expression as described in the above-mentioned application.

5. In furtherance of the present invention, a laboratory investigation was performed to study the comparative propensities of Response Genetics, Inc.'s ("RGI's") primers and other primers

for enhanced detection of the DPD markers. A description of the RGI primers used in this study and the results from the use thereof are found throughout the specification and particularly in Examples 2 and 3.

Furthermore, Figures 1 and 2 of the present invention illustrate comparisons of oligonucleotide primer pairs developed in-house at RGI for their ability to amplify DPD mRNA derived from various tissue samples. Moreover, these figures support the fact that the creation of primers must be such that the primers allow for enhanced levels of detection.

6. In Example 3 of the present invention, various primers were compared for ability to effectively detect DPD levels. It was found that the oligonucleotide primer pairs SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 were not effective in detecting DPD levels in various samples. It was discovered, however, that oligonucleotide primer pairs SEQ ID NO: 1 and SEQ ID NO: 2 and SEQ ID NO: 7 and SEQ ID NO: 8, when compared to existing primer sets, were more effective in accurately ascertaining DPD levels in tissue samples. These primers were more sensitive and enabled detection of extremely low levels of DPD expression in tissue samples.

7. To better illustrate the importance and need of oligonucleotide primers that allow for enhanced levels of detection, the oligonucleotide primers (and assay) of the present invention were compared against oligonucleotide primers produced by a research diagnostic company. In several of the DPD expression tests, the primers of the present invention were able to amplify the mRNA and provide a detectable result whereas the primers of the other manufacturer were not able to provide a result. (See table below.) Using the DPD primer pairs of the present invention, or those at least 80% identical thereto, RGI was able to provide reproducible results with

enhanced sensitivity, particularly in detection of low levels of DPD expression in a variety of tissue samples. The importance of detection of extremely low levels of DPD expression is discussed below.

Tissue Sample	**Value/ Detection using RGI primer of the present invention**	**Value/ Detection using Research Diagnostic Company primer**
CXF Section 1	0.004	undetectable
CXF Section 2	0.003	undetectable
CXF Section 3	0.002	undetectable
SW 62 Section 2	0.01	undetectable
SW 62 Section 4	0.01	undetectable
SW 62 Section 6	0.03	undetectable

Using the oligonucleotide primers of Example 3, as described in the present invention, the DPD expression was measured by the same method as in Example 3 in an embodiment of the present application. As discussed in the present application, it is vital to be able to detect patients with very low DPD levels undergoing 5-FU based therapy due to the potential of life-threatening toxicity. Thus, as illustrated by the data above, the oligonucleotide primers of the present invention are the most effective at detection of low levels of DPD in various tissue samples and provide a needed improvement over other DPD primers.

8. The undersigned declares that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United

States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12/23/04

Kathleen Danenberg